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MOLECULAR DETECTION OF SOILBORNE FUNGAL PATHOGENS OF CEREALS

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Introduction

Root diseases of wheat in eastern Oregon are often associated with a complex of pathogens, which may be composed of different species of *Rhizoctonia*, *Gaeumannomyces*, *Fusarium*, *Pythium*, nematodes, etc.. This confounds the symptoms and makes accurate identification difficult. As agriculture in the region shifts from conventional tillage to systems with less soil disturbance and alternate crops are introduced into the traditionally wheat/fallow regime, changes in the frequency, intensity and types of root diseases are expected. Rhizoctonia root rot, take-all, caused by *Gaeumannomyces graminis* variety *tritici* (Ggt) and Fusarium foot rot are among these. It is important to reduce the level of risk for disease, but this requires knowledge of the response of each pathogen to the new agronomic practices. This response involves interaction with the complex soil microbial community as it adjusts to changes in soil composition and management.

The long-term agronomic experimental plots at the Columbia Basin Agricultural Research Center (CBARC) offer a unique opportunity to investigate the effects of crop and soil management practices on wheat diseases. The following fungal pathogens are currently under investigation: *Rhizoctonia solani* AG 8, *R. oryzae*, *Gaeumannomyces graminis* var. *tritici* and var. *avenae* (Ggt and Gga), *Fusarium pseudograminearum* (formerly known as *F. graminearum* Group 1), *F. culmorum* and *F. graminearum* (Group 2). Molecular markers are currently available for each of these fungi. However, these markers vary in their specificity and sensitivity, which is a function of the segment of the genome they are designed to detect. This variability in specificity means that while a marker may be able to identify a laboratory isolate of a specific pathogen, it may not be able to positively identify the pathogen in soil. This is because soil contains many unknown organisms, and some may also give a positive result. Even if it is possible to identify a pathogen directly in soil, any correlation with, or prediction of, disease incidence and intensity is difficult, as it relies on the much less understood epidemiology of that particular disease. As research progresses on the development and application of species-specific molecular markers suitable for complex soil environments, more information will be obtained on disease dynamics. This knowledge, which includes interaction with other soil organisms, development of more aggressive strains and increased host range and effects of agronomic practices, soil type and climate on disease development, is the key to more sustainable disease control.

In our research, each molecular marker is being tested for its ability to identify and quantify the relevant pathogen directly in soil samples. Depending on the

results, markers judged to be suitable for soil detection are being used to assess the relationship of pathogen levels in the soil to disease expression in the different crop management systems at the CBARC. The objective is to identify agronomic practices that most effectively control a number of fungal diseases important in the Pacific Northwest.

Materials and Methods

Experimental Plots

Soil cores were removed from four long-term plots located at the CBARC in 2000: the grass pasture (GP) plot (established 1931), the continuous wheat (CW) conventionally tilled plot (1931), the no-till with added nitrogen and no-till without added nitrogen plots (1997), the crop residue (CR) conventionally tilled wheat/fallow rotation plot (1931), and an 18 year and a 3 year no-till wheat/fallow system (NTW). Eight to ten, 2 cm x 10 cm, cores were removed from the GP and CW plots and from each specified treatment of the CR and NTW plots. Soil samples were also taken from a *Fusarium* tolerance screening trial. For comparison, soil was removed from farmers' fields and an experimental plot near Ralston in Washington. Where possible, soil cores were taken close to plants within rows in order to obtain some root material, and these were combined, dried at 60^{o}C, ground to a fine powder and stored at -80^{o}C. Disease ratings for *Rhizoctonia* root rot were based on percentages of crown roots with lesions: 0 = none, 1 = <25 percent, 2 = 26-50 percent, 3 = 51-75, percent and 4 = >76 percent according to Smiley et al. (1992).

DNA Extraction

DNA was extracted from at least two 0.5 g sub-samples of soil using a soil DNA isolation kit (MoBio Laboratories Inc.) and combined. The kit was also used to extract DNA from isolates of different *Rhizoctonia solani* AGs, *Rhizoctonia oryzae*, *Gaeumannomyces graminis* varieties *tritici* (Ggt), *avenae* (Gga) and *graminis* (Ggg), *Fusarium pseudograminearum*, *F. culmorum, F. graminearum*, as well as other common soil fungal species and wheat roots. All DNA samples were quantified by UV spectrophotometry and quality was assessed by agarose gel electrophoresis.

Molecular Detection

The polymerase chain reaction (PCR), using primers specific to the *R. solani* AG 8 cloned sequence, pRAG12, (Brisbane et al. 1995, Matthew et al. 1995), nuclear ribosomal DNA small subunit (18S rDNA) of Ggt and Gga (Fouly and Wilkinson 2000), internal transcribed spacer (ITS) regions of *R. oryzae* (Mazzola et al. 1996), β-tubulin gene of *F. pseudograminearum* (Aoki and O'Donnell 1999) and a sequence characterized amplified region or SCAR (random amplified polymorphic DNA or RAPD derived) from *F. culmorum* (Schilling et al. 1996) was used to test for the presence of these fungi in soil samples. Reaction conditions were optimized for 10 to 20 ng[1] of soil DNA in a volume of 25 µl. Purified fungal DNA was used as a control and amplification products were separated by electrophoresis on an agarose gel. *R. solani* AG 8 was also measured in soil samples

[1] Units: fg (femtogram) = 10^{-15} g, pg (picogram) = 10^{-12} g, ng (nanogram) = 10^{-9} g, µg (microgram) = 10^{-6} g, µl (microliter) = 10^{-6} liters.

using the slot blot assay. Approximately 4 µg of soil DNA, which is the amount extracted from 1 g of soil, plus a set of AG 8 DNA standards were loaded onto a nylon-backed nitrocellulose membrane through individual wells of a slot blot apparatus (Bio-Rad Laboratories). The DNA was fixed onto the membrane by UV cross-linking and incubated at 68°C overnight in hybridization buffer containing DIG (digoxigenin)-labeled pRAG12 as the AG 8 probe. Hybridization of the probe to homologous DNA on the membrane was detected using chemiluminescence and visualized on X-ray film.

Results

R. solani AG 8 Detection

Test PCRs using primers for the pRAG12 AG 8-specific cloned sequence established that the 377 base pair (bp) DNA product (shown by arrows) was synthesized for this pathogen only, and not for a number of other common soil fungal pathogens (Fig. 1).

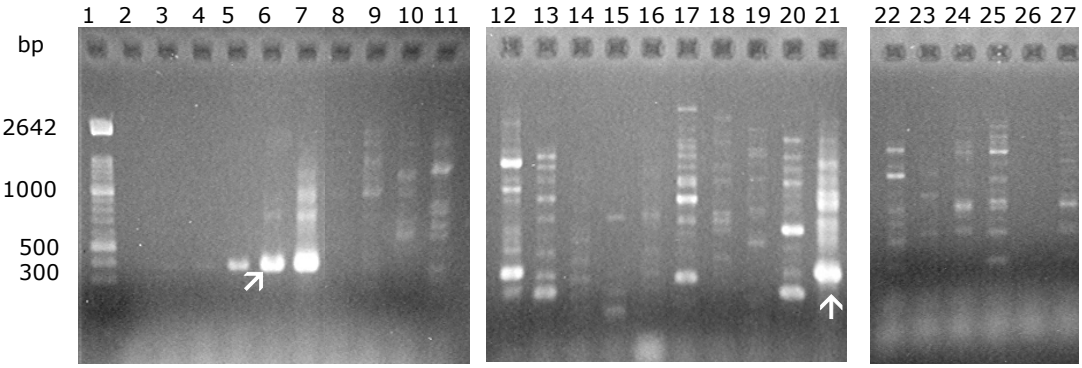

Figure 1. Amplification of fungal DNA with the AG 8-specific pRAG12 primers.
DNA in lanes 1-7: 100 bp molecular weight ladder, 0, 1fg, 10fg, 100fg, 1pg, 10pg AG 8; lanes 8-11: *F. pseudograminearum*, *F. graminearum*, *F. culmorum* and *F. avenaceum*; lanes 12-21 contain the following Rhizoctonias: AG D, unknown binucleate isolate, bridging isolate, *R. oryzae*, AG 2-1, AG 2-2, AG 3, AG 4, AG 6 GV and AG 8; lanes 22-27: *M. nivale*, *Bipolaris*, Gga, Ggg, *Pythium* and Ggt.

The addition of 10 ng of soil DNA (not containing the fungus) to purified AG 8 DNA gave only a slight reduction in band intensity after PCR. Therefore, pRAG12-primed PCR appeared to be a suitable method for detection of *R. solani* AG 8 in soil samples. However, it was not possible to quantify the amount of fungus present in the soil using this method. Figure 2 shows an example of PCR detection of *Rhizoctonia* AG 8 DNA in soil from some of the CR treatments. The location of the 377 bp AG 8 product is indicated by arrows. However, the simultaneous production of other weaker DNA bands indicates that the primers also amplify DNA from unknown sources (e.g., microorganisms present in the soil). A summary of the AG 8 PCR data for all the CBARC plots investigated so far, as well as available disease ratings and yield, is presented in Table 1.

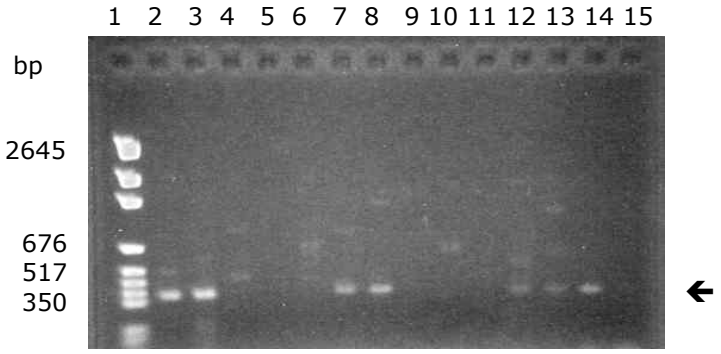

Figure 2. PCR detection of *R. solani* AG 8 in soil from the crop residue plot at the CBARC, Oregon, in 2000. Lane 1: pGEM molecular weight marker; Lanes 2-15: soil DNA from CR treatments.

Table 1. Detection of *R. solani* AG 8 in the crop residue (CR), continuous wheat (CW), grass pasture (GP) and 18 and 3 year no-till wheat/fallow (NTW) plots at the CBARC, Oregon, in 2000 using PCR, disease rating and crop yield.

Plots	Treatments	Yield[1] (bu/acre)		PCR - AG 8		Disease - Crown roots[2]	
2000		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
CR	Spring burn + 80lb/acre N	60	58.3	+	+	N/D[3]	
	No-burn + 80lb/acre N	64.3	65.3	-	-	N/D	
	Fall burn + no N	34	36.6	+	+	N/D	
	Spring burn + no N	29.1	31.2	+	+	N/D	
	No-burn + manure (10tons/acre)	78	88.2	-	-	N/D	
	No-burn + no N	35.2	41.4	+	+	N/D	
CW	No-till + no N	21.4[4]	N/A[3]	+	N/A	N/D	
	No-till + 102lb/acre N	84.2	N/A	+	N/A	1.1	N/A
	Tilled + no N	39.9	N/A	+	N/A	1.3	N/A
GP	Not grazed	N/A		+	N/A	N/A	
NTW	18 year no-till + no N	42.8	45.1	+	+	0	0
	18 year no-till + 120lb/acre N	116	122	+	-	0.8	0.4
	3 year no-till + no N	39	54.7[4]	+	+	0.2	0.2
	3 year no-till + 120lb/acre N	115.5	N/D	+	N/D	0.2	N/A
	Tilled + no N	65.2	64.4	-	+	0	0.2
	Tilled + 120lb/acre N	115	116.4	-	-	0.1	0.2

[1] Yield is the average of two replicates.
[2] Mean disease rating = total score/total number of plants. The seminal roots gave lower or zero values.
[3] N/A = not applicable; N/D = not measured.
[4] Treatment contained a bare patch caused by *R. solani* AG 8.

AG 8 was not detected in soil from either the no-burn + manure treatment or the no-burn + nitrogen treatment of the CR plot in 2000. These treatments had the highest

yield and visibly healthy wheat. AG 8 was detected at low to moderate levels in all other CR treatments. However, despite the presence of AG 8 in soil, no bare patches indicative of the disease were observed. AG 8 was also detected at low to moderate levels in GP soil, the NTW plot where indicated, and soil from the three CW experiments. While AG 8 was detected in all no-till treatments tested (CW and NTW) the disease rating was low. Disease was not assessed for the CW no-till + no N treatment, which contained a bare patch but was determined to be 0.2 for the NTW 3 year no-till + no N treatment, which also contained a bare patch. However, this patch was not in the area examined for yield or disease and the low disease rating may not provide a true assessment. There was no apparent correlation between the presence of the pathogen in the soil and the presence of diseased roots.

The PCR results were inconsistent between runs. In order to be confident that the absence of a 377 bp product signified absence of the pathogen, at least three separate reactions with the same or different soil DNA extractions were carried out for each sample. Since the variability obtained for the PCRs may be due to inhibitors in the soil DNA extractions or very low amounts of soil DNA used in the reaction, compounded by equally low levels of the AG 8 pathogen in the soil, the slot blot detection system was used in an attempt to obtain consistent results. The slot blot assay utilizes much larger amounts of DNA that would help overcome the quantity problem and is less susceptible to inhibitors. Figure 3 shows a slot blot of soil DNA from various plots at the CBARC plus a number of samples from farmers' fields. The intensity of the signal in each test slot was compared to that of the AG 8 standards for quantification of the amount of *Rhizoctonia* AG 8 in 4 µg soil DNA (equivalent to approximately 1 g soil). Using chemiluminescence/X-ray film for detection, 1 ng of AG 8 DNA (about 2,000 cells) was sufficiently above background to be used as a minimum detection level for soil testing.

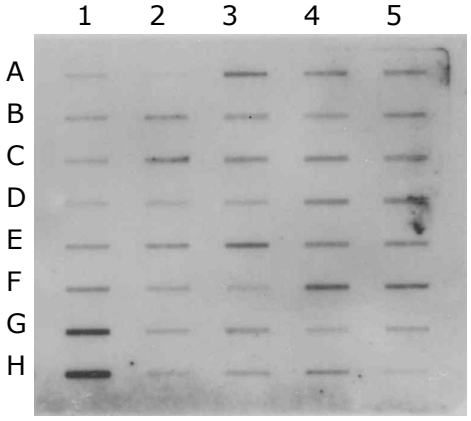

Figure 3. Slot blot assay of *R. solani* AG 8 in 4 µg of soil DNA (~1 g soil) using DIG-labeled pRAG12 DNA and chemiluminescence/X-ray film detection. The description of each sample is by column number and row letter:

1A – H: 50 pg, 100 pg, 500 pg, 1 ng, 5 ng, 10 ng, 50 ng and 100 ng *R. solani* AG 8 DNA. Wheat DNA was added to make each sample up to 100 ng total DNA.
2 A: 100 ng wheat DNA.
5 H: 100 ng *R. oryzae* DNA.

Remaining slots contain 4 µg soil DNA as follows:
2 B – H: Spring (Sp) and summer (S) 2000 CBARC - two bare patches (B42 and AWNT); Sp B42E, Sp B42I, S B42I, Sp AWNTI, S AWNTI, S B42O, S AWNTO (E = edge, I = inside, O = outside)
3 A – C: Summer 2000 Ralston WA; 403W, 302W, 401W in wheat.
4 A – C: Summer 1999 Ralston WA; 401W - inside, edge, outside patch in barley.
5 A – C: Summer 1999 Ralston WA; 304E - inside, edge, outside patch in spring wheat.
3 D – H: Summer 1999 Dusty WA; patch A outside, edge, inside; patch B inside, patch C inside.
4 D – E: Summer 2000 CBARC lupin plot, inside patch and general.
4 F – G: Summer 2000 farmer's field near WSU, Block 1 and Block 2.
4 H: Spring 2000 CBARC grass pasture.
5 D – G: Spring (Sp) and summer (S) 2000 CBARC - Sp CW00T, Sp CW00NT, S CW00T, S CW00NT.

In general, a higher level of AG 8 was detected in soil sampled in the spring, when plants were young, than in summer after harvest. In spring, levels of AG 8 ranged from ~10 ng AG 8 DNA/g soil in GP (4H) to ~30 ng AG 8 DNA/g soil in the CW tilled plot (5F), which did not display bare patching. However, similar levels were obtained for two bare patches, (2E) and (2B,C), with ~5 ng AG 8 DNA/g soil and 20 ng AG 8 DNA/g soil, respectively. Samples from Dusty WA (3E), Ralston WA (3A) and Block 1 of a farmer's field near Washington State University (WSU) (4F), which displayed high disease levels, also gave high levels (~30 ng AG 8 DNA/g soil). AG 8 was also detected at medium levels in the lupin plot (4D,E) and most of the Ralston plots.

Rhizoctonia oryzae Detection

The *R. oryzae* ITS primers were not found to be specific, but this fungus could be resolved from *R. solani* AG 8 and other fungal isolates using the banding patterns generated in a PCR. Figure 4A (top part of gel) shows that a prominent 511 bp band (solid white arrow) was produced for the four *R. oryzae* isolates in lanes 2-5, whereas a number of different sized DNA fragments resulted for *R. solani* AG 8 (lane 7). *R. oryzae* could not be detected in soil due to nonspecific amplification of DNA from unknown sources, which resulted in a smear overlaid with more prominent bands (lanes 8 and 9). The AG 8 pRAG12 primers did not amplify *R. oryzae* DNA, but identified the AG 8 isolate (open arrow, Figure 4B).

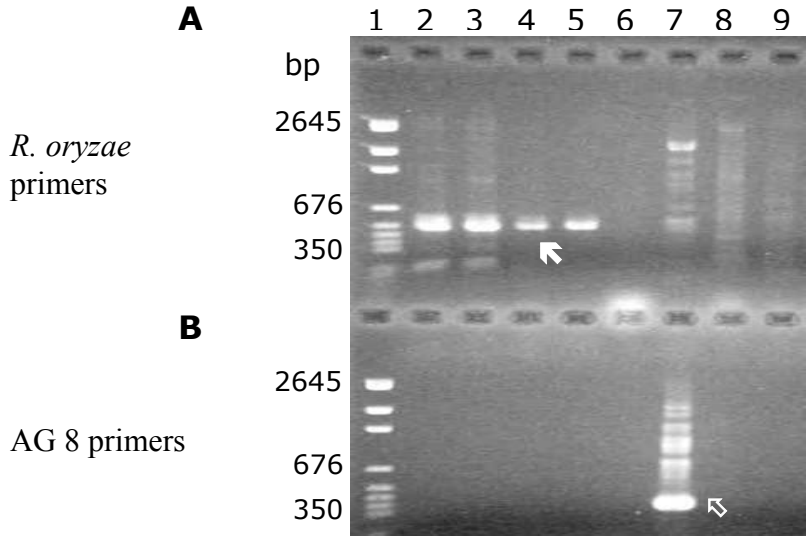

Figure 4. Amplification of *R. solani*, *R. oryzae* and soil DNA using the ITS primers for *R. oryzae* (A) and the pRAG12 primers for AG 8 (B). Top and bottom of gel: lane 1: pGEM molecular weight marker, lanes 2-5, *R. oryzae* DNA, lane 7: *R. solani* AG 8 DNA, lane 6: wheat DNA and lanes 8 and 9: soil DNA from the CBARC and a farmer's field.

Ggt and Gga Detection

Gaeumannomyces graminis variety *tritici* (Ggt) isolates could be resolved from Gga, Ggg and other fungi by the production of a single 410 bp DNA fragment in PCRs using the ribosomal DNA primers, NS5 and GGT-RP, shown in Figure 5 with a solid white arrow. The primers also amplified a Gga-specific 300 bp fragment (open arrow).

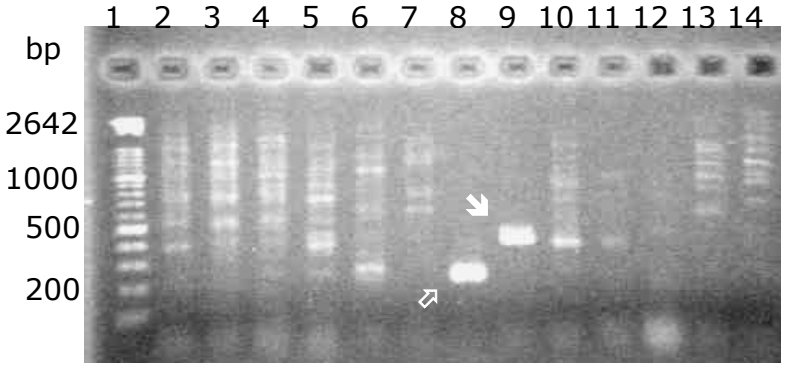

Figure 5. Amplification of Ggt, Gga, *Fusarium*, wheat and soil DNA with the NS5/GGT-RP primers. DNA in lane 1: 100 bp molecular weight ladder, lanes 2-5: soil, lane 6: wheat, lane 7: Ggg, lane 8: Gga, lane 9: Ggt, lanes 10-12: soil, lanes 13 and 14: *Fusarium pseudograminearum* and *F. graminearum*.

The production of a large number of fragments for soil DNA prevented positive identification of Ggt or Gga (lanes 2-5 and 10-12, Fig. 5), which is similar to the results obtained for the *R. oryzae* primers. Further analysis is being carried out to determine whether the Ggt and Gga associated band sizes can be detected in the smear produced for soil DNA. Two isolates, previously identified as Ggt on the basis of disease symptoms and colony morphology, did not produce the 410 bp band (results not shown). The occurrence of Ggt-like fungi in soil and on roots is well known and the PCR assay provides a reliable method for positive identification of both Ggt and Gga on diseased root material.

Fusarium Detection

The *F. pseudograminearum*-specific β-tubulin primers amplified the characteristic 523 bp fragment from *F. pseudograminearum* isolates only and not from other *Fusarium* species, *Rhizoctonia*, *Gaeumannomyces*, *Bipolaris*, or wheat (Fig. 6A and B, white arrows). In addition, a number of *Fusarium* isolates previously identified as *F. culmorum*, *F. graminearum*, *F. sambucinum*, *F. reticulatum* and *F. avenaceum* (Fig. 6A, lanes 5-9) on the basis of spore or colony morphology and color were confirmed as *F. pseudograminearum*. One isolate, which did not provide a band (Fig. 6A, lane 4) tested positive for the *F. culmorum* primers (results not shown).

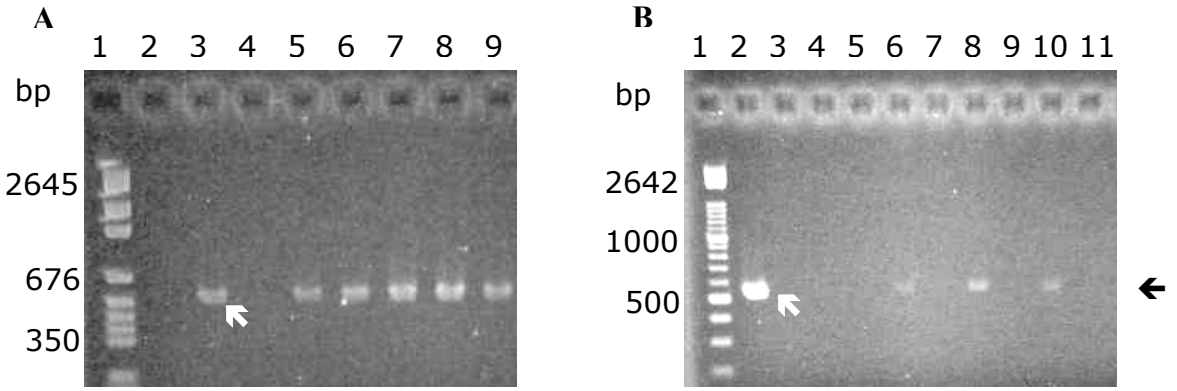

Figure 6. Amplification of *Fusarium* DNA with the *F. pseudograminearum* β-tubulin primers. DNA in (A) lane 1: pGEM marker, lane 2-4: *Bipolaris, F. pseudograminearum*, *F. culmorum* and lanes 5-9: unknown *Fusarium* isolates. (B) lane 1: 100 bp ladder, lanes 2-5: *F. pseudograminearum*, *F. graminearum*, *F. culmorum* and *F. avenaceum*, lanes 6-11: inoculated and un-inoculated wheat lines Stephens, Bruehl, and Connie.

In addition, the β-tubulin primers successfully detected strains of *F. pseudograminearum* in soil from three plots that had been inoculated with the fungus in the spring of 2000 (Fig. 6B, black arrow). However, since very low amounts of the product were made, it appeared that the pathogen was not present at high levels in

the soil at the time of sampling, just prior to harvest. Wheat cultivars were assessed by Dr. R. W. Smiley for tolerance on the basis of the number white heads. Connie, a durum wheat, had a very high number of white heads compared to Stephens (soft white winter wheat) and Bruehl (club wheat) and appears, therefore, to be less tolerant to the fungus than the other two varieties. The PCR-based DNA test showed that *F. pseudograminearum* was associated with all three cultivars in the inoculated plots regardless of disease expression as white heads (Fig. 6B, lanes 6, 8, 10). No correlation between the amount of 523 bp product synthesized and the level of tolerance to *F. pseudograminearum* was observed. However, as stated previously, due to limitations of the PCR assay, it is not possible to equate the amount of product made to the amount of fungus in the soil sample.

Discussion

Both the *R. solani* AG 8-specific pRAG12 sequence and its primers were used successfully to detect the fungus in soil by slot-blot and PCR assays, respectively. However, it was concluded that the slot-blot assay was more reliable at determining the presence and levels of AG 8 in soil. Since a larger amount of soil DNA, equivalent to a larger amount of soil, is analyzed, it increases the probability of detection. This is especially relevant to out-of-season summer soil collections when the fungus is at low levels naturally.

The results show that AG 8 is endemic to eastern Oregon and Washington, occurring at low levels in the grass pasture plot and in many farmers' fields in different cropping and climatic systems. The presence of *Rhizoctonia* in grass pasture may indicate a potential problem for land coming out of the Conservation Reserve Program (CRP). The molecular results obtained for the various plots tested at the CBARC showed no correlation between the presence or levels of AG 8 in the soil and expression of the disease as a patch. Therefore, the level of AG 8 DNA in soil may not be a good indicator of disease potential.

Rhizoctonia is a fungus that reacts quickly to agronomic and environmental changes. Temporal and spatial variability of the soil and pathogen make the disease patchy, and patchy low pathogen levels are difficult to monitor. It is possible that low levels of *R. solani* AG 8 observed in this region cause an overall reduction in plant health and consequent yield. Since this is most likely driven by agronomic practices that result in poor plant growth, and AG 8 was found in some high yielding plots, it suggests that certain land management practices can reduce the levels and/or effect of this fungus.

The results from this study indicate that two of the CR treatments and some no-till experiments may demonstrate suppressiveness to *R. solani* AG 8. In these cases the fungus was either present or absent in soil, disease ratings were low, there were no bare patches and the yields were high. This is in contrast to research conducted from 1989 to 1991, when the disease incidence and severity was apparently higher (Smiley et al. 1994). However, it is difficult if not impossible to compare the visual disease ratings taken approximately 10 years ago with the current assessments, due to their subjective nature and staff changes.

Since the symptoms produced by AG 8 can be confused with those caused by other

pathogens, a DNA-based test would confirm its presence on diseased roots, but it cannot provide an estimate of severity. Research in 2001 will concentrate on determining which agronomic practice from the CR, NTW, and CW plots at the CBARC is most effective at controlling root rot caused by *R. solani* AG 8. The slot blot technique will be used to quantify the levels of the fungus in the soil, plants will be assessed for diseased roots, and crop yields taken.

The ITS and rDNA primers for *R. oryzae* and Ggt/Gga, respectively, cannot be used in a soil PCR assay due to non-specific amplification of soil DNA. However, the assay may be applicable to the diagnosis of diseased plant roots. The development of more specific, high copy markers for both *R. oryzae* and *G. graminis* varieties *tritici* and *avenae* would facilitate the detection and quantification of these pathogens in soil samples. Similar to *R. solani* AG 8, DNA-based assays could be used to determine which agronomic systems most effectively control these pathogens.

The β-tubulin primers for *F. pseudograminearum* and the SCAR primers developed for *F. culmorum* can be used in PCRs for the diagnosis of diseased plant material and identification of isolates. However, since very low amounts of the single diagnostic fragment for *F. pseudograminearum* was produced in soil DNA samples known to contain the fungus, it may not be possible to use the β-tubulin-primed PCR assay for routine field soil tests. Because the soil was sampled very late in the season, just prior to harvest and after white heads had formed, it is possible that the pathogen is naturally present at low levels in the soil around the root zone at this time. This would concur with the biology of *F. pseudograminearum*, which carries over from season to season on infected plant debris originating from infected crown and basal stem tissue. Sampling at other times during the growing season needs to be done to determine whether a soil assay is feasible. Studies on the molecular detection of *F. culmorum*, which forms chlamydospores that can survive freely in the soil, also need to be carried out. It may also be necessary to develop more suitable markers for the detection of *Fusarium* species in soil.

The relationship between soil pathogens and the expression of disease is complex and influenced by many factors, including virulence and distribution of the pathogen, host susceptibility, agronomic practices, soil type, the environment, and competition, parasitism, inhibition and predation by other soil organisms. DNA-based diagnostic tests, using highly specific and sensitive markers, do offer improved identification over traditional plant pathological methods in the detection of pathogens in plant roots or soil. Pathogen-specific markers are useful for determining whether a particular pathogen is part of a disease complex, for which traditional methods of identification are unsuccessful or require much more time. A disease complex may exhibit confusing symptoms, both aerially and in the root zone, and it cannot always be certain which pathogen was the initial cause of the problem. Also, similar to the traditional methods, it does not provide the answers on why and how the disease developed or what to do to prevent it. The most important and long-term application of molecular markers specific to plant pathogens is for studies on the behavior of the organisms under different agronomic systems in different environments. Pathogens are a component of the soil microbial population. Knowledge of the

biology of each pathogen in soil, combined with a molecular/population analysis of the microbial diversity as influenced by the various agronomic practices and environment, will provide essential information required for the development of agronomic practices that reduce the potential for disease. The research being undertaken here is an attempt to identify the agronomic practices that most effectively control a number of fungal diseases important in the Pacific Northwest with the aim of sustainable disease control.

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References

Aoki, T., and K. O'Donnell. 1999. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91:597-609.

Brisbane, P.G., S.M. Neate, C.E. Pankhurst, N.S. Scott, and M.R. Thomas. 1995. Sequence-tagged site markers to identify *Rhizoctonia solani* AG 4 or 8 infecting wheat in South Australia. Phytopathology 85:1423-1427.

Fouly, H.M., and H.T. Wilkinson. 2000. Detection of *Gaeumannomyces graminis* varieties using polymerase chain reaction with variety-specific primers. Plant Disease 84:947-951.

Matthew, J., Herdina, and D.L. Whisson (Melanson). 1995. DNA probe specific to *Rhizoctonia solani* anastomosis group 8. Mycological Research 99:745-750.

Mazzola, M., O.T. Wong, and R.J. Cook. 1996. Virulence of *Rhizoctonia oryzae* and *R. solani* AG-8 on wheat and detection of *R. oryzae* in plant tissue by PCR. Phytopathology 86:354-360.

Schilling, A.G., E.M. Möller, and H.H. Geiger. 1996. Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. Phytopathology 86:515-522.

Smiley, R.W., A.G. Ogg, and R.J. Cook. 1992. Influence of glyphosate on Rhizoctonia root rot, growth and yield of barley. Plant Disease 76:937-942.

Smiley, R.W., H.P. Collins, P.E. Rasmussen, W. Uddin, K.L. Rhinhart, and R.H. Goller. 1994. Wheat diseases and yield in long-term agronomic experiments at the Columbia Basin Agricultural Research Center (Pendleton). Columbia Basin Agricultural Research Center Special Report 933:21-30.